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4.8. Peptides and Fibrils Stability in Water and Human Plasma

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Functionalized Peptide Fibrils as a Scaffold for Active Substances in Wound Healing

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Detailed protocol

This protocol was prepared to determine the preliminary bioavailability of the designed peptides in two types of medium. The stability studies were undertaken in human plasma and in water.

Materials and Reagents:

- trichloroacetic acid (TCA, Cas Number: 76-03-9, POCH)
- Water MiliQ (resistivity 18.18 mΩ·cm at 25 °C, conductivity 0.05 μS/cm pH 6.85)
- Peptide (prepared in-house)
- Human plasma (collected from healthy anonymous donors)
- Trifluoroacetic acid (TFA, Cas Number: 76-05-1, SigmaAdrich)

Equipment

- Eppendorf LowBind tubes 1.5 mL; 0.5 mL (Eppendorf AG)
- Tips LoRetention 1000 and 200 μL (Eppendorf AG)
- ThermoMixer™ C (Eppendorf AG)
- Pipets Research Plus: 20-200 μL and 100-1000 μL (Eppendorf AG
- Vortex Reax top (Heidolph)
- Ultrasonic bath (Bandelin Sonorex)
- Centriguge: Microfuge 16, (Beckman Coulter)
- UH-PLC system (Shimadzu)
- Mass spectrometer: ESI-IT-TOF (Shimadzu)
- Vials (Witko)

Medium preparation

1. Blood was collected from healthy anonymous donors and EDTA was then used as an anticoagulant. The procedure was approved by the Independent Bioethics Commission for Research of the Medical University of Gdańsk (NKBBN/387/2014).
2. Pass water with the specified physicochemical parameters through a 0.2 μL filter.

Peptide preparation – stock solution

1. Weigh 1 mg of the peptides in 1.5 mL Eppendorf LowBind tube, add 500 μL of the MiliQ water to final concentration 2 mg/mL.
2. If the solution is not clear, mixing the peptide for 1 min using Vortex and then place it

Chromatographic solutions:

1. Composition of solution A and B for chromatographic analyses :

A – 2000 mL water MiliQ with 2.0 mL TFA
B – 1600 mL Acetonitrile with 400 mL water MiliQ and 1.6 mL TFA

15 % TCA:

1. Dissolve 1.5 g of TCA in 8.5 mL MiliQ water, mixed with Vortex

Procedure:

1. Take 210 μL of the peptide from the stock solution (C_{stock} = 2 mg/mL) and transfer it to a fresh LowBind Eppendorf tube with a volume of 1.5 mL. Add 210 μL of either human plasma or MiliQ water, depending on the type of analysis, and mix by Vortex for 1 minute. The final concentration of the samples should be 1 mg/mL.
2. Incubate all samples in 37 °C with agitation 300 rpm in the Thermomixer. Analyze the samples after 0, 1, 2, 3, 6 and 24 h.
3. Take 80 μL of the incubated samples at the appropriate time point and transfer them to the fresh LowBind tube with a volume of 500 μL. Add 20 μL of the 15 % trichloroacetic acid (TCA) , to stop the reaction and precipitate plasma proteins.
4. Incubate the mixed samples in covered ice for 10 minutes. After, centrifuge the samples for 10 minutes at 12,000 rpm in a Microfuge.
5. To analyze the supernatant, transfer 80 μL into vials and use an analytical column, such as Kinetex 2.6 μm C18 100 Å, 100 x 4.6 mm (Shimadzu), in the RP-HPLC system.
6. All samples must be analyzed under the following conditions: injection volume of 20 μL, a linear gradient of acetonitrile (B solution) ranging from 5 % to 100 % over 15 minutes, and detection at 223 nm. To determine the extent of peptide degradation in the analyzed sample, compare the peak area to that of the reference sample.
7. To confirm the presence of the peptide and analyze any resulting degradation fragments, use LC-MS spectrometer to analyze the sample obtained in step 6.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Sawicka, J. , Iłowska, E. , Deptuła, M. , Sosnowski, P. , Sass, P. , Czerwiec, K. , Chmielewska, K. , Szymańska, A. , Pietralik-Molińska, Z. , Kozak, M. , Sachadyn, P. , Pikuła, M. and Rodziewicz-Motowidło, S. (2023). 4.8. Peptides and Fibrils Stability in Water and Human Plasma. Bio-protocol Preprint. bio-protocol.org/prep2206.
2. Sawicka, J., Iłowska, E., Deptuła, M., Sosnowski, P., Sass, P., Czerwiec, K., Chmielewska, K., Szymańska, A., Pietralik-Molińska, Z., Kozak, M., Sachadyn, P., Pikuła, M. and Rodziewicz-Motowidło, S.(2021). Functionalized Peptide Fibrils as a Scaffold for Active Substances in Wound Healing. International Journal of Molecular Sciences 22(8). DOI: 10.3390/ijms22083818